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# Phylogenomics, introgression, and demographic history of South American true toads (*Rhinella*)

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## Abstract

The effects of genetic introgression on species boundaries and how they affect species' integrity and persistence over evolutionary time have received increased attention. The increasing availability of genomic data has revealed contrasting patterns of gene flow across genomic regions, which impose challenges to inferences of evolutionary relationships and of patterns of genetic admixture across lineages. By characterizing patterns of variation across thousands of genomic loci in a widespread complex of true toads (*Rhinella*), we assess the true extent of genetic introgression across species thought to hybridize to extreme degrees based on natural history observations and multilocus analyses. Comprehensive geographic sampling of five large-ranged Neotropical taxa revealed multiple distinct evolutionary lineages that span large geographic areas and, at times, distinct biomes. The inferred major clades and genetic clusters largely correspond to currently recognized taxa; however, we also found evidence of cryptic diversity within taxa. While previous phylogenetic studies revealed extensive mitonuclear discordance, our genetic clustering analyses uncovered several admixed individuals within major genetic groups. Accordingly, historical demographic analyses supported that the evolutionary history of these toads involved cross-taxon gene flow both at ancient and recent times. Lastly, ABBA-BABA tests revealed widespread allele sharing across species boundaries, a pattern that can be confidently attributed to genetic introgression as opposed to incomplete lineage sorting. These results confirm previous assertions that the evolutionary history of *Rhinella* was characterized by various levels of hybridization even across environmentally heterogeneous regions, posing exciting questions about what factors prevent complete fusion of diverging yet highly interdependent evolutionary lineages.

**KEYWORDS**
demographic modelling, hybridization, introgression, phylogenomics, *Rhinella*

## 1 | INTRODUCTION

How introgression affects reproductive isolation and speciation is an enduring question in evolutionary biology. Reproductive isolation has long been viewed as the primary factor behind lineage divergence and stable boundaries between closely related species (Avise et al., 1998; Mayr, 1963; Rabosky, 2016). When closely related populations come into contact, however, gene flow via hybridization can lead to the introgression of alleles (Mallet, 2005; O'Connell et al., 2021). Introgression levels can vary starkly across genome regions.

  

In particular, in the presence of strong divergent selection, those loci underlying adaptive phenotypes can maintain marked differentiation even with extensive gene flow among closely related populations (Feder et al., 2012). Thus, these varying degrees of isolation across the genome may contribute to the maintenance of species boundaries despite the homogenizing effects of gene flow (Yeaman & Whitlock, 2011).

Differential introgression across genomic regions can lead to dramatic topological discordance between genealogies inferred from distinct genes, as illustrated by instances of mitonuclear discordance (Bernardo et al., 2019; Bessa-Silva et al., 2020; Firneno et al., 2020). This gene-tree heterogeneity must be accounted for as it can make reconstructing evolutionary relationships and historical demography challenging (Carstens & Knowles, 2007; Firneno et al., 2020; Liu et al., 2010). The increasing availability of high-throughput sequencing data sets for non-model organisms has improved our ability to discern patterns of introgression in closely related species or populations (Firneno et al., 2020; Graham et al., 2018; Lavretsky et al., 2016) and thus clarify phylogenetic relationships and species limits. This is especially so in large, widely distributed species complexes with limited variation in external morphological traits and hybridization blurring species limits (Guo et al., 2016; Phuong et al., 2017; Potter et al., 2016) .

The increasing availability of genome-scale data sets has also fostered the development of model-based approaches to infer historical demographic events such as population size shifts and pulses of gene flow (Portik et al., 2017; Prates, Xue, et al., 2016). These approaches have transformed our understanding of how landscape and climate changes have contributed to the assembly of regional species pools, for instance by limiting dispersal, promoting speciation, or leading to lineage fusion (Graham et al., 2018; Lavretsky et al., 2016; Leaché et al., 2019; Portik et al., 2017). One flexible approach involves simulating population histories to compare the fit of empirical genome-scale data to data simulated under alternative biogeographical scenarios (Portik et al., 2017; Prates et al., 2016; Dal Vechio et al., 2019). This modelling framework can facilitate hypothesis testing, such as how climate-driven habitat shifts may have led to migration, introgression, or isolation across geographic regions. These approaches have been instrumental to shed light on the historical factors behind present-day spatial biodiversity patterns in regions that concentrate large proportions of biodiversity. This is the case of the Neotropics, where demographic inference has supported that Late-Quaternary climate fluctuations and Neogene geomorphological change have played a major role in shaping species range limits, genetic diversity levels, and lineage divergence (Gehara et al., 2017; Pirani et al., 2020; Prates, Xue, et al., 2016). Nevertheless, biogeographic investigations in the Neotropics have often shown geographic and taxonomic bias, which questions the generality of the mechanisms invoked to explain species richness and distributions. For instance, taxa with wide ranges across South America's open vegetation biomes – the dry and highly seasonal Cerrado, Caatinga, and Chaco – have received relatively less attention than rainforest biotas (Fonseca et al., 2018; Gehara et al., 2017; Werneck, 2011).

One example of a Neotropical clade whose biogeographic history remains poorly known is the true South American toads, genus *Rhinella* (Bufonidae). Despite being the focus of a handful of phylogeographic studies, the evolutionary relationships and species limits between these toads remain elusive, perhaps due to wildly varying patterns of introgression and hybridization across species (Maciel et al., 2010; Pereyra et al., 2016, 2021; Sequeira et al., 2011; Vallinoto et al., 2010). As such, not only the evolutionary history of this group is unclear, but so are the environmental and geographic factors that may have favoured introgression and its variation, or how hybridization may have contributed to lineage divergence or fusion (Azevedo et al., 2003; Correa et al., 2012; Malone & Fontenot, 2008; Pereyra et al., 2016; Sequeira et al., 2011). *Rhinella* is composed of multiple species complexes that are each distributed across much of the Neotropics. These groups are known to harbour high levels of cryptic lineage diversity, as revealed by single and multilocus genetic analyses (Maciel et al., 2010; Pereyra et al., 2016, 2021; Vallinoto et al., 2010). Among them is the *Rhinella marina* group, best known for the globally invasive species *R. marina*. Previous studies of this group have identified both mitochondrial and nuclear introgression across species (Azevedo et al., 2003; Maciel et al., 2010; Vallinoto et al., 2010). However, lack of data about persisting genetically admixed populations in the wild makes it difficult to assess the magnitude of presumed hybridization and how it affects species boundaries (Azevedo et al., 2003; Malone & Fontenot, 2008; Pereyra et al., 2021). Despite the ecological diversity seen in *Rhinella*, with taxa that span savannas, rainforests, and xeric shrublands, biogeographic analyses have largely focused on taxa occurring within a single biome (Sequeira et al., 2011; Thomé et al., 2010), which is also the case of other South American anuran clades (Fonseca et al., 2018; Gehara et al., 2017; Oliveira et al., 2018). As a result, how habitat transitions may contribute to patterns of gene flow and species range limits remains unclear.

In this investigation, we focus on the *R. marina* group to investigate evolutionary relationships, quantify the extent of hybridization, and examine whether landscape transitions among South America's biomes impose limits to gene flow and species ranges. For this purpose, we focus on *R. marina*, *R. poeppigii*, *R. horribilis*, *R. jimi*, and *R. schneideri* (also known as *R. diptycha*), which have established contact zones throughout the continent. We infer population structure, gene flow, and relationships based on geographically comprehensive sampling of genomic variation within each taxon. We then proceed to test alternative historical hypotheses to quantify plausible demographic events such as population size shifts and historical gene flow. With this approach, we seek to answer the following questions: what are the levels of genetic structure across and within each species? Do genomic data corroborate a pattern of widespread admixture or introgression across these species, as previously suggested based on only a few loci? Lastly, what historical demographic processes may explain species distributions and genetic diversity patterns within this clade?

## 2 | MATERIALS AND METHODS

### 2.1 | Sample collection

Our sampling included 191 individuals belonging to the *Rhinella marina* species group, as follows: 72 *R. marina*, 39 *R. schneideri*, 23 *R. horribilis*, 11 *R. jimi*, and nine *R. cf. poeppigii*, as well as four *R. veredas*, eight *R. rubescens*, and 25 *R. icterica* which were used in the mitochondrial analysis only. We also included samples from the *Rhinella granulosa* and *R. margaritifera* major clades within *Rhinella* as outgroups in the divergence time estimation analyses (see below). Within each species in the focal group consisting of *R. marina*, *R. horribilis*, *R. schneideri*, *R. jimi*, and *R. poeppigii*, we sample multiple individuals from each locality across their known ranges, with the exception of *R. cf. poeppigii*, which was identified as distinct from *R. marina* a posteriori based on the genetic data (see Results) (Acevedo et al., 2016; Maciel et al., 2010; Vallinoto et al., 2010). Tissue samples were obtained from the MTR herpetological tissue collection hosted at Instituto de Biociências, University of São Paulo (IBUSP) with vouchers at Museum of Zoology, University of São Paulo, as well as from the Amphibian and Reptile Diversity Research Centre (ARDRC), and the Louisiana State University Museum of Natural Science (LSUMNS) (Table S1).

### 2.2 | DNA extraction, amplification and sequencing

We extracted genomic DNA using a standard phenol-chloroform extraction protocol (Sambrook & Russell, 2006). Fragments of the mitochondrial 16S were amplified using 16Sar and 16Sbr primers and sequenced on an ABI 3730xL (primer information and PCR conditions in the Text S1). Sequences were edited and aligned in Geneious Prime 2020.0.4 (identification and accession numbers in Table S1). We generated double-digest restriction-site associated DNA sequencing (ddRADseq) data following (Peterson et al., 2012), with modifications as described in Streicher et al. (2014). Briefly, 200–500 ng of DNA were digested using the *Sbf*I (restriction site 5′-CCTGCAGG-3′) and *Msp*I (restriction site 5′-CCGG-3′) restriction enzymes in a single reaction using the manufacturer's recommended buffer (New England Biolabs) for 5 h at 37°C. Digested DNA was bead-purified before ligating barcodes and index adaptors, then samples with the same index were pooled and size-selected (415–515 bp) on a Blue Pippin Prep size selector (Sage Science). Final Library preparation was analysed and quantified on a BioAnalyser (Agilent) and Qubit Fluorometer 4 (Thermo Fisher Scientific). The resulting 100 bp single-end libraries were sequenced at MedGenome on an Illumina HiSeq2500.

We used the command line version of ipyrad v. 0.9.45 (Eaton & Overcast, 2020) (available at https://ipyrad.readthedocs.io) to demultiplex and assign reads to individuals based on sequence barcodes (allowing no mismatches from individual barcodes), perform reference read assembly (minimum clustering similarity threshold = 0.90), align reads into loci, and call single nucleotide polymorphisms (SNPs). As a reference, we used the *Rhinella marina* genome (Edwards et al., 2018). A minimum Phred quality score (= 33), sequence coverage (= 6×), read length (= 35 bp), and maximum proportion of heterozygous sites per locus (= 0.5) were enforced, while ensuring that variable sites had no more than two alleles (i.e., a diploid genome). Following the initial assembly, we used Matrix Condenser (de Medeiros & Farrell, 2018) to assess levels of missing data across samples and then reassembled our data set to ensure a minimum sample coverage of less than 35% missing loci within each sample and at least 75% of samples at each locus. This strategy resulted in a final data set composed of 49,376 SNPs at 3318 RAD loci with less than 12% missing data. Additionally, Weir and Cockerham mean $F_{ST}$ estimates for the ddRADseq data set using VCFTools (Danecek et al., 2011) and Nei's $G_{ST}$ for the mitochondrial data set were calculated using the R package mmod (Winter, 2012).

### 2.3 | Inferring population structure and genetic admixture

Based on the ddRAD data, we used a genetic clustering approach to estimate the number of demes and if admixture was present among them. We assembled a SNP data set as described above but excluding outgroups and using only one SNP per RAD locus to maximize sampling of independent SNPs. This approach resulted in a data set composed of 3314 SNPs. Genetic clustering was performed using the maximum likelihood method ADMIXTURE, testing up to 15 populations with 20 replicates per K and a 10-fold cross-validation (Alexander et al., 2009; Portik, 2016). The best K was determined by assessing the replicate with the lowest cross-validation error. To further characterize population structure, we used the nonparametric method of discriminant analysis of principal components (DAPC), implemented in the R package adegenet (Jombart & Ahmed, 2011; Jombart et al., 2010). The *find.clusters* function was used to test the fit of 1–15 clusters (K). The K with the lowest Bayesian information criterion (BIC) score was considered the best-fit number of demes. The resulting ancestry coefficient matrices (Q-matrices) were then imported into QGIS (QGIS Development Team, 2020, QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org) to make average-per-locality pie-charts indicating admixture levels at each sampled locality for each species.

### 2.4 | Phylogenetic analyses

We reconstructed maximum likelihood phylogenies for both the mitochondrial and the unlinked ddRADseq loci data sets using IQTREE v2.1.2, utilizing the built-in model selection tool ModelFinder Plus, implementing 1000 ultrafast bootstraps (Hoang et al., 2018; Kalyaanamoorthy et al., 2017; Nguyen et al., 2015). We specified that all partitions share the same branch lengths and selected the best-fit partitioning scheme by merging partitions (which implements

the greedy algorithm of PartitionFinder), testing the MrBayes' substitution model set and considering the top 10% partition schemes using the fast relaxed clustering algorithm from PartitionFinder2 to save computational time (Chernomor et al., 2016; Lanfear et al., 2012, 2014, 2017). In addition, we performed phylogenetic inference under a Bayesian framework for both data sets using MrBayes 3.2.6 (Ronquist et al., 2012), implementing three independent runs of four Markov chains of 10 million generations each and sampling every 1,000 generations with the first 25% generations discarded as burnin. We used Tracer 1.7 (Rambaut et al., 2018) to assess whether Markov chain mixing was adequate (effective sample sizes >200) and to visually assess model parameter stationarity and convergence between runs. We then summarized a 50% majority-rule consensus tree.

To estimate divergence dates and inform the delimitation of species boundaries, we conducted Bayesian divergence dating analyses based on the mtDNA data set in BEAST2 using an HKY model of nucleotide substitution, a log-normal relaxed molecular clock, and a coalescent constant population size prior. We follow Pramuk et al. (2008) by enforcing a minimum age for the root node between the *Rhinella marina* and *R. granulosa* species complexes based on a *Rhinella marina* fossil from the Clarendonian North American Stage of the middle Miocene (c. 11 Ma), described by Sanchiz (1998), and employed a log-normally distributed prior with a standard deviation of 0.5. We ran this analysis for 20 million generations sampling every 1,000 generations. Runs were assessed using TRACER v1.7 (Rambaut et al., 2018) to examine convergence. We then summarized a maximum clade credibility tree using TreeAnnotator discarding the first 25% of trees as burnin (Bouckaert et al., 2019; Stamatakis, 2014). All phylogenetic tree-based methods were analysed on Cipres (Miller et al., 2010).

## 2.5 | Demographic modelling with ∂a∂i

We used the diffusion-approximation method ∂a∂i (Gutenkunst et al., 2009) to test alternative hypotheses of population history within the *Rhinella marina* clade based on species ranges, previously reported potential hybridization events, common biogeographic patterns exhibited by amphibians across this region, as well as computational limitations. Using both two- and three-dimensional joint site frequency spectra (2D- and 3D-JSFS), we divided the data set into two population subsets: one comprised of *R. marina*, *R. horribilis*, and *R. jimi*; and another comprised of *R. schneideri* and *R. cf. poeppigii*. Folded-JSFS data sets were used in all ∂a∂i analyses.

We filtered the ddRAD data for each subset to allow no more than 35% missing data from any sample, removed singletons, and selected one SNP per locus using VCFtools (Danecek et al., 2011; Gutenkunst et al., 2009; Portik et al., 2017). We then used the *stacks_pipeline* Python script from Portik et al. (2017) to create the SNP input file for ∂a∂i. We used the python script *easySFS* (https://github.com/isaacovercast/easySFS) to determine the projection size of each population, which was determined by balancing a downscaled sample size that maximized the number of segregating sites (Gutenkunst et al., 2009; Marth et al., 2004). In ∂a∂i, we then tested a range of extrapolation grid sizes (40–100 in 10-unit increments, e.g., 50, 60, 70 to 100, 110, 120) in the divergence-with-no-migration model to determine the appropriate grid size by selecting the model with the highest log-likelihood, implementing four rounds of optimization totaling 100 replicates. Once an optimal grid size was determined, each tested model was run 3 times independently.

For the subset composed of *R. marina*, *R. horribilis*, and *R. jimi*, we used a 3D-JSFS to test models incorporating gene flow at different times, including those accounting for ancient migration, recent secondary contact, and past simultaneous divergence of all lineages (Figure S5). In addition to a model of (1) divergence with no migration, we tested the following models: (2) divergence with continuous symmetric gene flow between all populations, (3) divergence with continuous symmetric gene flow between geographically adjacent populations, (4) isolation followed by secondary contact, (5) simultaneous divergence in isolation followed by more recent secondary contact between adjacent populations, (6) simultaneous divergence with continuous symmetric migration between adjacent populations, (7) ancient migration with very recent isolation, (8) ancient migration with a longer period of recent isolation, (9) a short ancient period of migration followed by a long period of isolation, and 10) ancient migration followed by lineage isolation and population size change across two epochs (Barratt et al., 2018; Portik et al., 2017).

For the subset composed of *R. schneideri* and *R. cf. poeppigii*, we tested 2D-JSFS models incorporating differing migration levels at different time periods (Figure S6). In addition to a model of (1) divergence with no migration, we tested the following models: (2) divergence with continuous symmetric migration, (3) divergence with continuous asymmetric migration, (4) divergence with continuous symmetric migration and a varying rate of migration across two epochs, (5) divergence with continuous asymmetric migration and a varying rate of migration across two epochs, (6) divergence in isolation, followed by symmetric secondary contact, (7) divergence in isolation, followed by asymmetric secondary contact, (8) ancient symmetric migration then subsequent isolation, (9) ancient asymmetric migration then subsequent isolation, (10) divergence in isolation followed by symmetric secondary contact with subsequent isolation, and (11) divergence in isolation followed by asymmetric secondary contact with subsequent isolation (Charles et al., 2018; Portik et al., 2017).

Best-fit models were chosen based on log-likelihood values, which we assumed to be the true likelihood (and not composite likelihood) given that we have kept only one SNP per RAD locus. Replicates with the consistently highest likelihood scores were used to calculate and compare models using the Akaike information criterion (AIC).

## 2.6 | Inferring gene flow

To further explore potential hybridization between taxa, we inferred Patterson's D statistic, or the ABBA-BABA statistic, and the related admixture fraction estimates, or $f_4$-ratio statistics, based on the ddRAD

data using Dsuite (Malinsky et al., 2021; Patterson et al., 2012). Tests were designed with a 4-taxon fixed phylogeny (((P1,P2)P3)O), wherein a typical ancestral ("A") and derived ("B") allele pattern should follow BBAA. Under incomplete lineage sorting, conflicting ABBA and BABA patterns should occur in equal frequencies, resulting in a D statistic =0. If, however, introgression between P3 and P1 or P2 has occurred, there should be an excess of these patterns and a D statistic significantly different from 0, with significance detected using a block-jackknifing approach (Durand et al., 2011; Green et al., 2010; Malinsky et al., 2021; Patterson et al., 2012). We used the *f*-branch or $f_b$(C) metric to tease apart potentially correlated $f_4$-ratio statistics and estimate gene flow events between internal branches on the phylogeny (Malinsky et al., 2018; Martin et al., 2013). Dsuite uses a VCF file and a jackknifing approach to assess correlations in allele frequencies between closely-related species (Malinsky et al., 2021). Within Dsuite, we used the *Dtrios* and *Fbranch* programs to identify introgression between all combinations of species, as well as potential direction of gene flow, specifying *Rhinella veredas* as an outgroup and applying the Benjamini-Hochberg (BH) correction to control for the false discovery rate.

## 3 | RESULTS

### 3.1 | Phylogenetic relationships

The 16S phylogeny (160 individuals; 481 base pairs) suggested little phylogenetic structure within the *Rhinella marina* complex. One clade included most of the *R. horribilis* samples, while individuals from the remaining taxa formed a polytomy (Figure S1). Maximum likelihood and Bayesian phylogenies based on the ddRADseq data set (128 individuals) resulted in fully concordant phylogenies (Figure 1). These analyses inferred six highly supported clades, two corresponding to *R. marina* and the other four corresponding to *Rhinella schneideri*, *R. horribilis*, *R. jimi*, and a clade tentatively assigned to *R. cf. poeppigii* (BS = 100; PP = 1.0; Figures 1–3). These putative *R. poeppigii* samples were originally identified as *R. marina*, which would render *R. marina* to be paraphyletic; however, after re-examining these specimens morphologically, we were able to positively identify samples from western Amazonia in Brazil's state of Acre as *R. poeppigii*, while closely related samples from eastern localities in the state of Pará were morphologically more similar to *R. marina* (Figure S7). Pairwise Nei's $G_{ST}$ estimates for the 16S data were much lower than the Weir and Cockerham weighted $F_{ST}$ estimates for the ddRADseq data. Across all taxa, the average pairwise $G_{ST}$ for the mitochondrial data was 0.117 (0.025–0.228) while the average pairwise $F_{ST}$ for the nuclear data was 0.506 (0.379–0.843) (Table S2).

The time-calibrated phylogeny based on the 16S mitochondrial data dated the root of *Rhinella marina* at 5.58 Ma (95% HPD: 2.75–9.40 million years ago (Ma); Figure 4). Although many relationships had poor support due to lack of variability within the locus, some clades showed high support, including a clade with most of the *R. horribilis* samples, which was dated at 1.03 Ma (95% HPD: 0.35–2.04 Ma). Two samples not included in this clade were samples distributed in the northern Andes, which clustered with other *R. marina* samples (Figure 4). One highly supported clade consisted of two clades with a divergence date of 0.67 Ma (95% HPD: 0.18–1.48 Ma): one clade consisted of *Rhinella cf. poeppigii* samples from eastern Amazonia, and other consisted of the western Amazonia *R. poeppigii* sample as well as *R. marina* from southern Amazonia (Figure 4). Additionally, *R. granulosa* is estimated to be sister to the *R. marina* complex, with *R. margaritifera* more distantly related. Due to the lack of variation within the *R. marina* group, we interpret dates within this complex with caution.

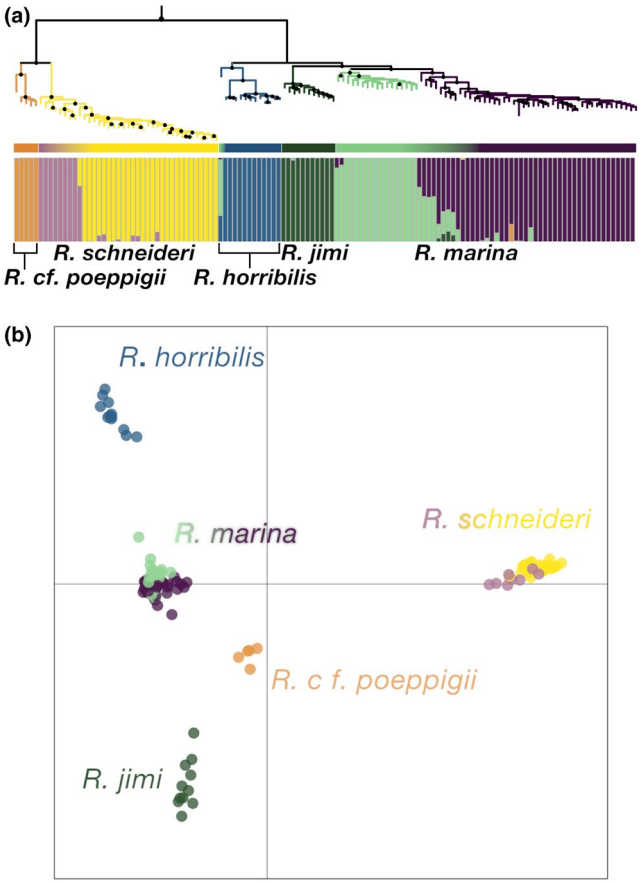


FIGURE 1 (a) Maximum likelihood phylogeny of *Rhinella marina* complex focal species using ddRADseq data and corresponding ADMIXTURE plot (*K* = 7). Black circles on the phylogeny denote ML bootstrap support (BS) >95 and Bayesian posterior probability (PP) >0.95. (b) DAPC plot (*K* = 7)

### 3.2 | Population structure

Despite the high posterior probabilities of each clade in our ddRAD tree, the ADMIXTURE results supported genetic admixture both within and across multiple taxa within the *Rhinella marina* complex (Figure 1), with a best-fit K of 7. Each ddRAD clade corresponded to a cluster, except for the *Rhinella schneideri* clade which consisted of two nonreciprocally monophyletic units. *Rhinella horribilis* (blue, Figures 1 and 2) showed admixture from the northern cluster of *R. marina* into one northern Andes locality. One cluster of *Rhinella marina* was relegated to northern Amazonia (light green, Figures 1 and 2), while

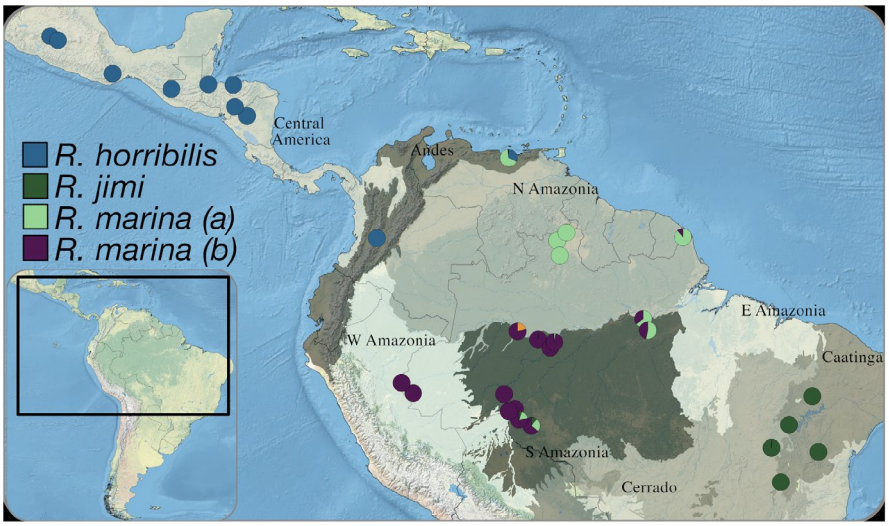


**FIGURE 2** Locality map for the subset depicting average ADMIXTURE cluster assignments per locality ($K = 7$) for *Rhinella horribilis*, *R. marina*, and *R. jimi*. Colors correspond to Figure 1. Map partitioned into biomes (Central America, Northern Andes, Northern Amazonia, Western Amazonia, Eastern Amazonia, Southern Amazonia, Pantanal, Chaco, Cerrado, Caatinga, Northern Atlantic Forest)

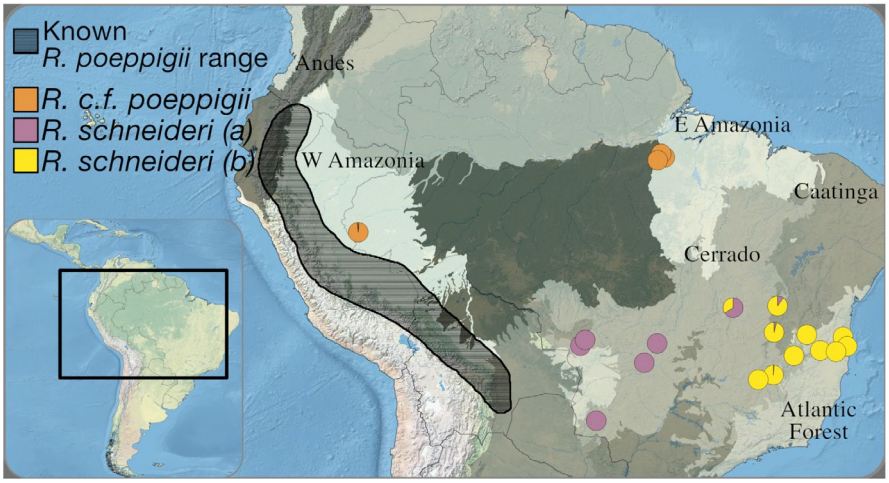


**FIGURE 3** Locality map for the subset depicting average ADMIXTURE cluster assignments per locality ($K = 7$) for *Rhinella poeppigii* and *R. schneideri*. Colours correspond to Figure 1. Map partitioned into biomes (Northern Andes, Northern Amazonia, Western Amazonia, Eastern Amazonia, Southern Amazonia, Pantanal, Chaco, Cerrado, Caatinga, Northern Atlantic Forest, Southern Atlantic Forest). *R. poeppigii* range adapted from De la Riva (2002); Venâncio et al. (2017); Venegas and Ron (2014)

the other cluster showed a cline of admixture across its western and southern Amazonia clades (light green to purple, Figures 1 and 2) and admixture from *R. jimi* and *R. cf. poeppigii* (dark green and orange, Figure 1). The two genetic clusters within *R. schneideri* (pink and yellow, Figures 1 and 3) followed an east-west admixture gradient across the Cerrado to the northern Atlantic Forest, as well as intermediate ecotones. *Rhinella jimi* occurs mostly in the semi-arid Caatinga shrublands of northeastern Brazil, but also in the adjacent coastal rainforest (dark green, Figures 1 and 2). The DAPC analysis supported this clustering scheme as well; however, BIC scores suggested similar support for six to eight clusters (Figure S4). The seven clusters recovered were concordant with phylogenetic structure (Figure 1).

### 3.3 | Demographic inference

For the subset composed of *R. marina*, *R. horribilis*, and *R. jimi*, the best 3D-JFSF model was one that incorporated ancient migration with a short period of recent isolation since divergence, with a log-likelihood of −1572.69 and AIC of 3165.38 (Figure 5, Table 1, Table S3). This model included an ancient period of migration between all lineages (mA, Figure 5), then another period of migration between geographically adjacent species after the divergence between *R. marina* and *R. jimi*, and then subsequent lineage isolation. Parameter estimates indicated a much longer ancient period of migration between all lineages with smaller migration rates (T1 = 10.82; mA = 0.05) compared to the shorter time of adjacent-species migration with higher rates of migration (T2 = 0.12; m1 = 1.36; m2 = 0.85) and the shortest period of isolation (T3 = 0.10) (Table 1, Table S3).

For the subset composed of *R. schneideri* and *R. cf. poeppigii*, the best 2D-JFSF model incorporated divergence in isolation followed by secondary contact with asymmetric gene flow, with a log-likelihood of −539.27 and AIC of 1090.54 (Figure 5, Table 1, Table S3). Parameter estimates inferred a period of divergence in isolation (T1 = 0.07) with a shorter period of secondary contact (T2 = 0.01) and a much higher rate of migration from *R. cf. poeppigii* into *R. schneideri* (m12 = 15.5) than from *R. schneideri* into *R. cf. poeppigii* (m21 = 1.82) (Table 1, Table S3).

### 3.4 | D-statistics

Nearly all topological trios tested (((P1,P2)P3)O) had significant *D*-statistics (Table S4), indicating that the majority of gene flow

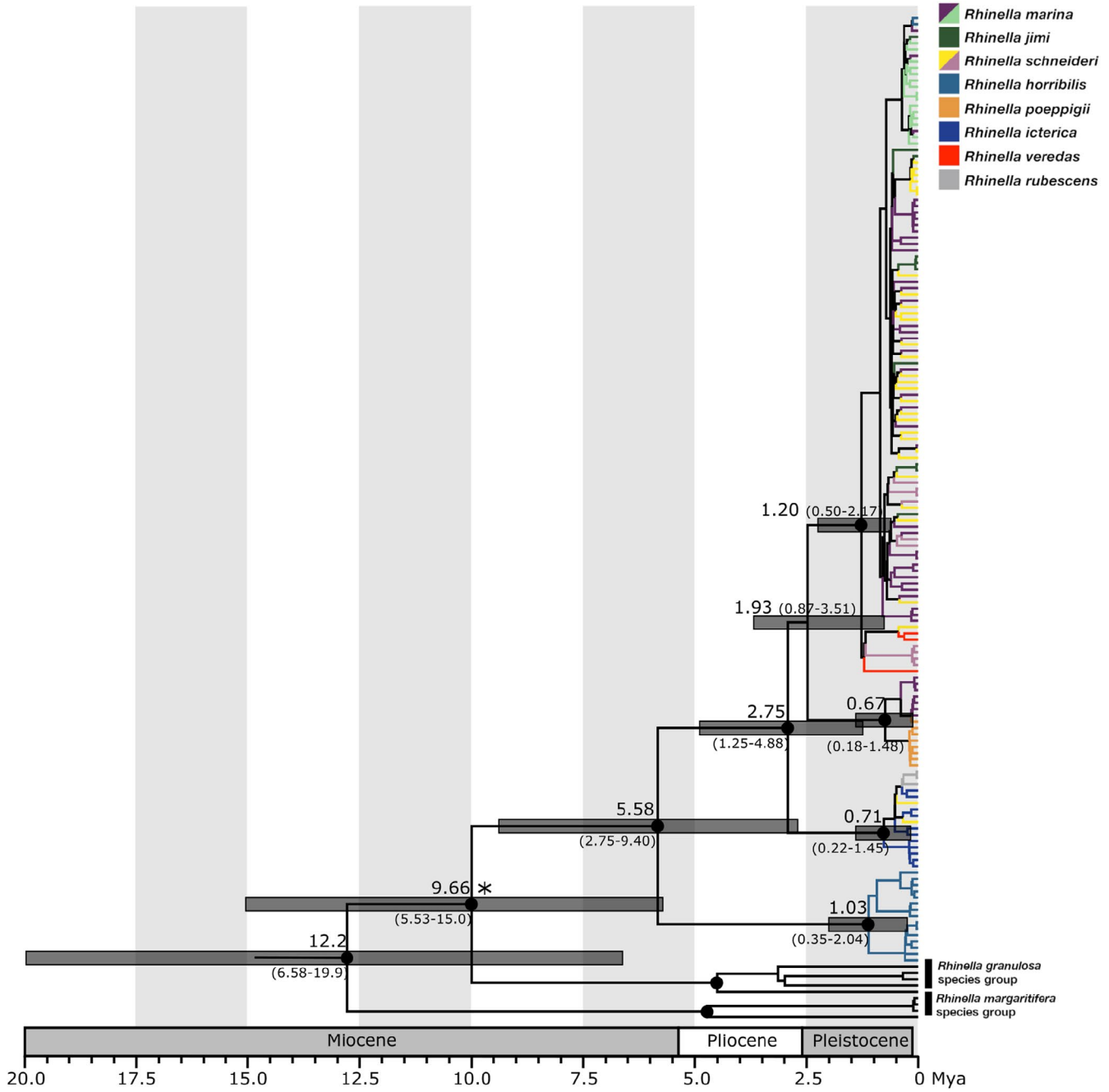


**FIGURE 4** Time calibrated phylogeny based on mitochondrial 16S data. Black circles indicate PP >0.95, * indicates the fossil calibration, and the bars represent the 95% HPD, which are also in parentheses. Colours correspond to the phylogeny in Figure 1

within this group is not due to incomplete lineage sorting. The *R. jimi-marina-horribilis* trio was not significant ($p > .05$), indicating that we could not reject the null hypothesis of no gene flow, with ABBA-BABA patterns arising solely due to incomplete lineage sorting (Malinsky et al., 2021). *D*-statistics for all significant trios ranged from 0.12 to 0.49 (Table S4). The highest *D*-statistics were for *R. horribilis-jimi-schneideri* (0.49), *R. marina-jimi-schneideri* (0.37), and *R. schneideri-poeppigii-marina* (0.30). The *fb*(C) statistic is a summary of *f*4 admixture ratios and shows excess allele sharing between the branch on the y-axis and the sample on the x-axis (Malinsky et al., 2018). The *fb*(C) statistics indicated the highest percentages of gene flow between *R. cf. poeppigii* and *R. marina* (11%), between *R. cf. poeppigii* and *R. horribilis* (8%), and between *R. jimi* and *R. schneideri* (7%) (Figure 6, Table S5).

## 4 | DISCUSSION

Based on comprehensive geographic and genomic sampling within a clade of South American toads, this investigation found evidence of multiple distinct evolutionary lineages that span large geographic areas and, at times, distinct biomes. The inferred major clades and

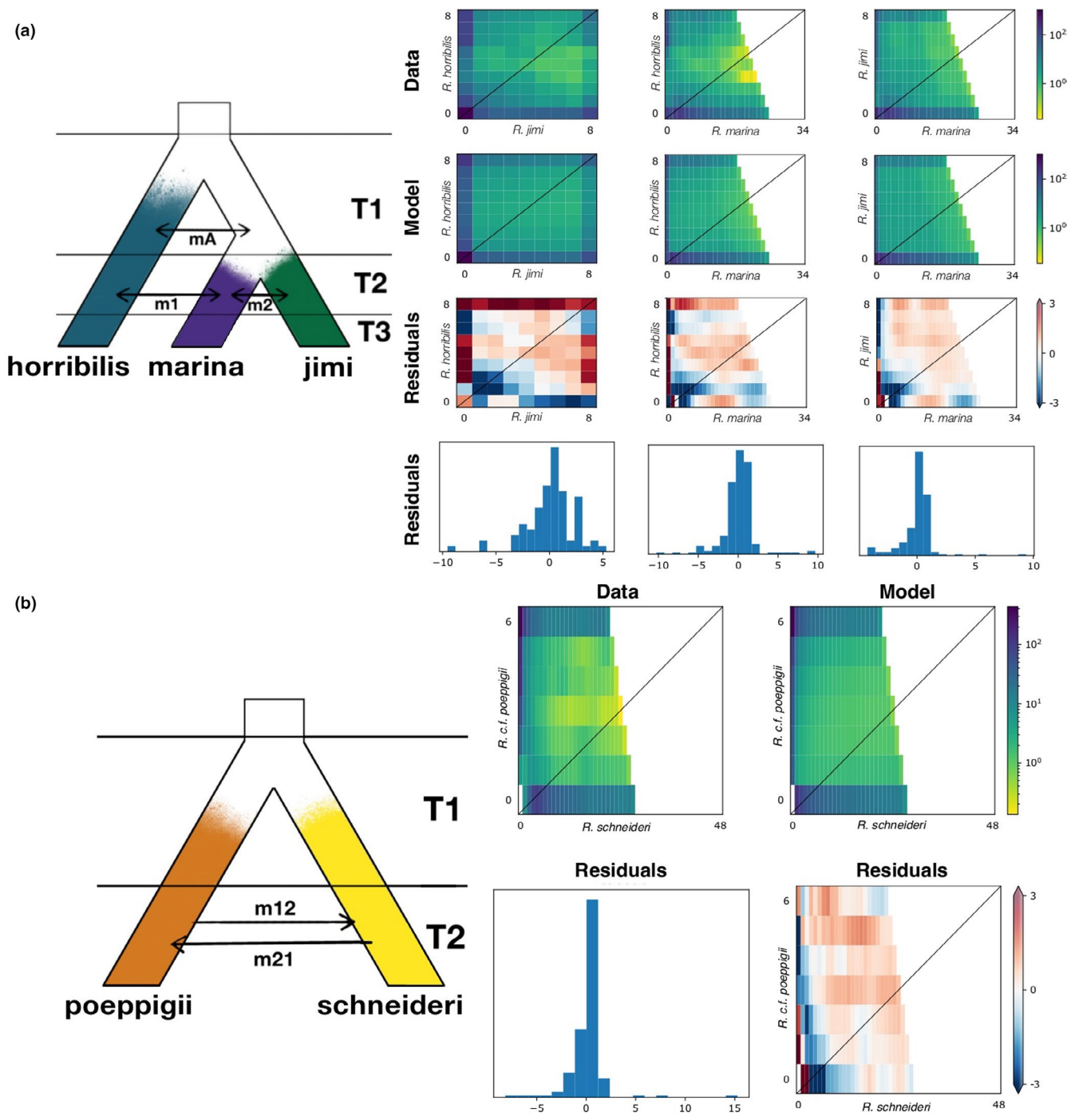


FIGURE 5 Optimal demographic models and residual plots for the (a) 3D-JSFS analysis of *Rhinella horribilis*, *R. marina*, and *R. jimi*, and (b) 2D-JSFS analysis of *R. poeppigii* and *R. schneideri*

genetic clusters largely correspond to currently recognized taxa within *Rhinella*; however, we also found evidence of potentially cryptic diversity within *R. marina*, *R. schneideri*, and potentially *R. poeppigii*. Genetic clustering analyses suggested that many of the inferred groups include admixed individuals. Accordingly, demographic analyses supported that the evolutionary history of these toads involved cross-taxon gene flow both at ancient (in the case of *R. marina*, *R. horribilis*, and *R. jimi*) and recent (in the case of *R. schneideri* and *R. cf. poeppigii*) times. Both demographic inference and ABBA-BABA tests inferred patterns of genetic introgression across species, supporting previous assertions that the evolutionary history of *Rhinella* was characterized by various levels of hybridization (Pereyra et al., 2016; Sequeira et al., 2011).

## 4.1 | Phylogenetic patterns and species boundaries

The phylogenetic findings of this study improve our knowledge about species diversity and distributions in South America. Our sampling validates previous reports of *Rhinella poeppigii* present in western

TABLE 1 Optimal demographic models and estimated parameters

| Model | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2D | LL | AIC | θ | nu1 | nu2 | | m12 | m21 | | | | T1 | T2 | | | |
| Divergence in isolation with continuous asymmetric secondary contact | −539.27 | 1090.54 | 1424.5 | 0.01 | 0.05 | | 15.5 | 1.82 | | | | 0.07 | 0.01 | | | |
| 3D | LL | AIC | θ | nu1 | nu2 | nu3 | nuA | mA | m1 | m2 | T1 | T2 | T3 | | | |
| Ancient migration with shortest isolation | −1572.69 | 3165.38 | 95.33 | 0.57 | 0.15 | 1.31 | 6.15 | 0.05 | 1.36 | 0.85 | 10.82 | 0.12 | 0.10 | | | |

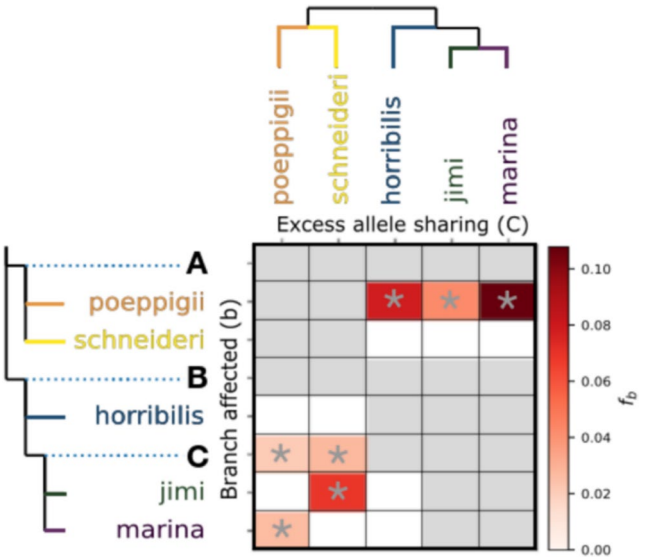


FIGURE 6 The $f_b$ statistic (summary of $f_4$ admixture ratios). Grey colour corresponds to tests that are not possible because of constraints on the phylogeny. *Indicates a significant result

Amazonia (Venâncio et al., 2017). *Rhinella poeppigii* has a history of both taxonomic uncertainty and misidentification, due to its similarity to *R. marina* (De la Riva, 2002; Venâncio et al., 2017; Venegas & Ron, 2014). After the first individuals were identified and collected in Ecuador, subsequent specimens collected in the region that were previously misidentified were discovered at Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ) (Venegas & Ron, 2014). In this study we included another individual from Porto Walter, Acre, Brazil, which further corroborates *R. poeppigii* extending into Brazil. Furthermore, we uncovered a group of *R. cf. poeppigii* specimens in eastern Amazonia near the Belo Monte Hydroelectric dam on the Xingu River (Figure 3). These samples, however, do not display distinct *R. poeppigii* morphology, and in fact are more similar morphologically to *R. marina*, to which they were originally assigned (Figure S7). Unfortunately, as sampling of this clade was initially unintentional, we sampled only a few individuals that could confidently be called *R. poeppigii* that did not occur across the range of *R. poeppigii*, which may be misrepresenting the genetic admixture visualized within this clade (Figures 1 and 3). Given this restricted sampling and the more than 2,000 km distance in sampled individuals, it may be that eastern *R. cf. poeppigii* is actually a yet undescribed cryptic species within the *Rhinella marina* complex.

The mitochondrial 16S rRNA marker has been used extensively for identification and barcoding of amphibians (Maya-Soriano et al., 2012; Rockney et al., 2015; Vences et al., 2005). Despite this marker being extremely useful in taxonomic identification for a number of closely related species (Firneno & Townsend, 2019), even within the *Rhinella* genus (Pereyra et al., 2016), there is an inherent lack of diversity recovered across all focal species within the *R. marina* complex (Figures S1 and S2). It is possible that purifying selection has acted on this region of the mitochondrial genome, thereby greatly reducing genetic diversity across the complex (Charlesworth et al.,

1995; Cvijović et al., 2018). Considering that processes like purifying selection can also reduce genetic diversity at linked neutral sites, previous estimates of potential introgression within *Rhinella* species using mitochondrial data may be similarly affected (Cvijović et al., 2018). This phenomenon could have resulted in an overestimation of shared loci by any other means, such as hybridization, as opposed to a constraint on particular loci. With the 16S fragment sequenced being relatively short (~480 bp), an analysis of the entire 16S rRNA gene or even the whole-mitochondrial genome in this group could prove useful in disentangling the reasons for such low genetic diversity seen here.

By contrast, despite evidence of admixture both within and between species, nuclear data estimated a phylogeny with substantial structure and support (Figure 1). When compared to other phylogenies generated with single or multilocus data sets, high-throughput sequencing of the *Rhinella marina* complex has revealed a surprising amount of genetic complexity, introgression, and interspecific resolution (Bessa-Silva et al., 2020; Maciel et al., 2010; Vallinoto et al., 2010). These patterns suggest that in groups with such complex demographic histories, and especially those with a likelihood of hybridization between divergent populations or species, large-scale genetic data can be very useful in disentangling relationships and histories. These types of data could be utilized in future studies to identify potentially adaptive regions of the genome that correlate with phenotypic or ecological differences between populations.

### 4.2 | Biogeographic drivers of species range limits

Inferred species range limits can be attributed to both present-day spatial environmental gradients and the history of topographic change in South America, as suggested for a number of other South American taxa (Carnaval et al., 2009; Fonseca et al., 2018; Gehara et al., 2014; Prates, Rivera, et al., 2016). Mitochondrial divergence time analyses are consistent with the idea that the Andean uplift contributed to divergence between *R. marina* and *R. horribilis* (Figure 4); pronounced genetic divergence between populations on each side of the Andean chain supports the recent recognition of *R. horribilis* as a taxon distinct from *R. marina* (Vallinoto et al., 2010). While the Andes probably limits contemporary gene flow between these two taxa, our finding of admixture between them suggests that the northern Andes may be a semi-permeable barrier (Figure 2), in agreement with patterns seen in other organisms (Acevedo et al., 2016; Bessa-Silva et al., 2020; Maciel et al., 2010). Additionally, like other amphibians (Noonan & Wray, 2006) and reptiles (Gamble et al., 2008), the extensive fluvial network formed in western Amazonia by periodic Miocene flooding, known as the Pebas formation, may have contributed to divergence not only between *R. horribilis* and *R. marina*, but also between the northeast and south-southwestern Amazonian clades within *R. marina* (Vallinoto et al., 2010; Wesselingh & Salo, 2006). *Rhinella marina*, which is comprised of two well-supported clades, is distributed across Amazonian climates, which are known to have asynchronous historical eastern-western climatic cycles and have had an effect on species composition and genetic diversity within the biome (Cheng et al., 2013; Prates, Rivera, et al., 2016). Considering that the distinct clades have a northern-southern distribution, as opposed to an eastern-western distribution, however, it may be more plausible that geographic barriers, such as fluctuating fluvial networks from the Miocene through the Pleistocene (Cooke et al., 2012; Lundberg et al., 1998), have had a higher impact in promoting divergence between these clades within *R. marina*.

Similar to what is observed within *R. marina*, we see patterns of species distributed across environmental gradients repeated across the phylogeny; *R. schneideri* is distributed across the Cerrado, through Cerrado-Caatinga-Atlantic Forest ecotones, and into the northern Atlantic Forest, with an east-west gradient of admixture (Figures 1 and 3). The Seasonally Dry Tropical Forests and savannas of South America have been known to harbor complex and cryptic genetic diversity and have been especially affected by Quaternary climate fluctuations (Bandeira et al., 2021; Fonseca et al., 2018; Gehara et al., 2017; Prado et al., 2012; Vasconcellos et al., 2019; Werneck et al., 2015). Considering the phylogenetic pattern that we see within *R. schneideri*, we can posit that this species expanded eastward during Plio-Pleistocene climate change (Bandeira et al., 2021; Lisiecki & Raymo, 2007). Paleoclimatic modelling of the biogeographic history and niche of *R. schneideri* on a finer scale is recommended to validate this hypothesis.

A puzzling biogeographic pattern that emerged from our results is the extremely disjunct distribution between *R. poeppigii* in western Amazonia and its sister clade, *R. cf. poeppigii*, from eastern Brazilian Amazonia, more than 2,000 km apart. This mysterious pattern has also been reported for other herpetofaunal species, including the lizards *Anolis trachyderma* (Ribeiro-Júnior, 2015) and *Potamites ecpleopus* (Ribeiro-Júnior & Amaral, 2017) and the horned treefrog *Hemiphractus scutatus* (de Lima Moraes & Pavan, 2018). Despite this large geographic distance, as well as the effects of contrasting climatic seasonality between the eastern and western localities in this region on other herpetofauna (Cheng et al., 2013; Prates, Rivera, et al., 2016; Wang et al., 2017), this and other studies indicate limited genetic divergence across disjunct regions (de Lima Moraes & Pavan, 2018). A comprehensive analysis of museum specimens and available tissues from these areas, in conjunction with a more thorough sampling of *R. poeppigii* across its range, will be required to confirm this unexpected pattern of genetic divergence within this group.

### 4.3 | Hybridization and introgression

The interspecific relationships inferred with historical demographic modelling suggest extremely varied patterns of migration and hybridization through time within the *Rhinella marina* complex. Our study indicates that species within this group have diverged across multiple biomes and amassed significant genetic differentiation despite continuous gene flow among species (Figure 5).

Many of the species within the *Rhinella marina* complex also have a shared introgressive history (Figures 1 and 6; Tables S4 and S5). Hypothesis testing of demographic models suggests that the *R. marina-horribilis-jimi* clade continued to exchange genes throughout its dispersion across the continent, and species within this clade exchanged genes with other species within the complex (Figures 1, 5 and 6, Table S5). *Rhinella* species have long been observed to have overlapping ranges with the potential for hybridization, especially given the propensity for these toads to participate in "mating balls", wherein different toad species – and sometimes from different genera – seemingly mate with one another (Fontenot et al., 2011; Maciel et al., 2010; Pereyra et al., 2016; Sequeira et al., 2011; Thomé et al., 2012). There is a relative lack of data, however, surrounding behavioral aspects of intraspecific interactions among species outside of these mating balls. Despite evidence of gene flow between species, there was no evidence of population-wide hybridization or the presence of hybrid species within our sampling. Potential proposed hybridization events have been reported within or between *Rhinella* species groups, such as within the *R. granulosa* complex (Guerra et al., 2011; Pereyra et al., 2016) and the *R. crucifer* complex (Júnior et al., 2004; Thomé et al., 2012), where either instances of morphologically intermediate individuals or hybrid populations have been reported. Much of the speculation surrounding hybridization in Neotropical toads has been accompanied by a lack of data from natural populations to assess the biological reality of presumed hybrid species (Fontenot et al., 2011; Malone & Fontenot, 2008; Thomé et al., 2012). Within the *R. marina* group, however, we found that recurrent gene flow between species at low levels is much more prevalent than the persistence of repeated gene flow in massive multispecies mating events.

## CONFLCIT OF INTEREST

The authors have no conflict of interest to declare.

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## AUTHOR CONTRIBUTIONS

Danielle Rivera and Matthew K Fujita conceived and designed the study. Danielle Rivera, Matthew K Fujita, Miguel Trefaut Rodrigues and Ivan Prates acquired funding. Miguel Trefaut Rodrigues, Ivan Prates, and Janalee P. Caldwell provided samples. Danielle Rivera, Ivan Prates, and Thomas J. Firneno wrote software used for analysis. Danielle Rivera and Thomas J. Firneno analysed the data. Danielle Rivera, Ivan Prates, and Matthew K Fujita wrote the manuscript with input from all authors.

## DATA AVAILABILITY STATEMENT

16S sequences and demultiplexed genetic data have been made available through NCBI (BioProject PRJNA772164; Table S1) and associated scripts and input files have been made available through dryad: https://doi.org/10.5061/dryad.7pvmcvdtp.

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## REFERENCES

Acevedo, A. A., Lampo, M., & Cipriani, R. (2016). The cane or marine toad, *Rhinella marina* (Anura, Bufonidae): two genetically and morphologically distinct species. *Zootaxa*, *4103*(6), 574–586.

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*(9), 1655–1664. https://doi.org/10.1101/gr.094052.109

Avise, J. C., Walker, D., & Johns, G. C. (1998). Speciation durations and Pleistocene effects on vertebrate phylogeography. *Proceedings Biological Sciences/The Royal Society*, *265*(1407), 1707–1712.

Azevedo, M. F. C., Foresti, F., Ramos, P. R. R., & Jim, J. (2003). Comparative cytogenetic studies of *Bufo ictericus*, *B. paracnemis* (Amphibia, Anura) and an intermediate form in sympatry. *Genetics and Molecular Biology*, *26*(3), 289–294. https://doi.org/10.1590/S1415-47572003000300012

Bandeira, L. N., Villalobos, F., Werneck, F. P., Peterson, A. T., & Anciães, M. (2021). Different elevational environments dictate contrasting patterns of niche evolution in Neotropical Pithecopus treefrog species. *Biotropica*, *53*, 1042–1051. https://doi.org/10.1111/btp.12929

Barratt, C. D., Bwong, B. A., Jehle, R., Liedtke, H. C., Nagel, P., Onstein, R. E., Portik, D. M., Streicher, J. W., & Loader, S. P. (2018). Vanishing refuge? Testing the forest refuge hypothesis in coastal East Africa using genome-wide sequence data for seven amphibians. *Molecular Ecology*, *27*(21), 4289–4308. https://doi.org/10.1111/mec.14862

Bernardo, P. H., Sánchez-Ramírez, S., Sánchez-Pacheco, S. J., Álvarez-Castañeda, S. T., Aguilera-Miller, E. F., Mendez-de la Cruz, F. R., & Murphy, R. W. (2019). Extreme mito-nuclear discordance in a peninsular lizard: The role of drift, selection, and climate. *Heredity*, *123*(3), 359–370. https://doi.org/10.1038/s41437-019-0204-4

Bessa-Silva, A., Vallinoto, M., Sampaio, I., Flores-Villela, O. A., Smith, E. N., & Sequeira, F. (2020). The roles of vicariance and dispersal in the differentiation of two species of the *Rhinella marina* species complex. *Molecular Phylogenetics and Evolution*, *145*, 106723. https://doi.org/10.1016/j.ympev.2019.106723

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., & Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *15*(4), e1006650.

Carnaval, A. C., Hickerson, M. J., Haddad, C. F. B., Rodrigues, M. T., & Moritz, C. (2009). Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. *Science*, *323*(5915), 785–789.

Carstens, B. C., & Knowles, L. L. (2007). Estimating species phylogeny from gene-tree probabilities despite incomplete lineage sorting: an example from *Melanoplus* grasshoppers. *Systematic Biology*, *56*(3), 400–411. https://doi.org/10.1080/10635150701405560

Charles, K. L., Bell, R. C., Blackburn, D. C., Burger, M., Fujita, M. K., Gvoždík, V., & Portik, D. M. (2018). Sky, sea, and forest islands: diversification in the African leaf-folding frog *Afrixalus paradorsalis* (Anura: Hyperoliidae) of the Lower Guineo-Congolian rain forest. *Journal of Biogeography*, *45*(8), 1781–1794. https://doi.org/10.1111/jbi.13365

Charlesworth, D., Charlesworth, B., & Morgan, M. T. (1995). The pattern of neutral molecular variation under the background selection model. *Genetics*, *141*(4), 1619–1632. https://doi.org/10.1093/genetics/141.4.1619

Cheng, H., Sinha, A., Cruz, F. W., Wang, X., Edwards, R. L., d'Horta, F. M., Ribas, C. C., Vuille, M., Stott, L. D., & Auler, A. S. (2013). Climate change patterns in Amazonia and biodiversity. *Nature Communications*, *4*, 1411. https://doi.org/10.1038/ncomms2415

Chernomor, O., von Haeseler, A., & Minh, B. Q. (2016). Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology*, *65*(6), 997–1008. https://doi.org/10.1093/sysbio/syw037

Cooke, G. M., Chao, N. L., & Beheregaray, L. B. (2012). Marine incursions, cryptic species and ecological diversification in Amazonia: The biogeographic history of the croaker genus *Plagioscion* (Sciaenidae). *Journal of Biogeography*, *39*(4), 724–738. https://doi.org/10.1111/j.1365-2699.2011.02635.x

Correa, C. L., Méndez, M. A., Veloso, A., & Sallaberry, M. (2012). Genetic and reproductive evidence of natural hybridization between the sister species *Rhinella atacamensis* and *Rhinella arunco* (Anura, Bufonidae). *Journal of Herpetology*, *46*, 568–577. https://doi.org/10.1670/10-266

Cvijović, I., Good, B. H., & Desai, M. M. (2018). The effect of strong purifying selection on genetic diversity. *Genetics*, *209*(4), 1235–1278. https://doi.org/10.1534/genetics.118.301058

Dal Vechio, F., Prates, I., Grazziotin, F. G., Zaher, H., Grabosky, R., & Rodrigues, M. T. (2019). Rain forest shifts through time and riverine barriers shaped the diversification of South American terrestrial pit vipers (*Bothrops jararacussu* species group). *Journal of Biogeography*, *47*(2), 516–526. https://doi.org/10.1111/jbi.13736

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A. & 1000 Genomes Project Analysis Group (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

De la Riva, I. (2002). Taxonomy and distribution of the South American toad *Bufo poeppigii* Tschudi, 1845 (Amphibia, Anura, Bufonidae). *Graellsia / Editada Por El Instituto Espanol De Entomologia Del Consejo Superior De Investigaciones Cientificas*, *58*(1), 49–57. http://dx.doi.org/10.3989/graellsia.2002.v58.i1.266

de Lima Moraes, L. J. C., & Pavan, D. (2018). On the occurrence of Hemiphractus scutatus (Spix, 1824) (Anura: Hemiphractidae) in eastern Amazonia. *Amphibian & Reptile Conservation*, *12*(1), 5–14 (e151).

de Medeiros, B. A. S., & Farrell, B. D. (2018). Whole-genome amplification in double-digest RADseq results in adequate libraries but fewer sequenced loci. *PeerJ*, *6*, e5089. https://doi.org/10.7717/peerj.5089

Durand, E. Y., Patterson, N., Reich, D., & Slatkin, M. (2011). Testing for ancient admixture between closely related populations. *Molecular Biology and Evolution*, *28*(8), 2239–2252. https://doi.org/10.1093/molbev/msr048

Eaton, D. A. R., & Overcast, I. (2020). ipyrad: Interactive assembly and analysis of RADseq datasets. *Bioinformatics*, *36*(8), 2592–2594. https://doi.org/10.1093/bioinformatics/btz966

Edwards, R. J., Tuipulotu, D. E., Amos, T. G., O'Meally, D., Richardson, M. F., Russell, T. L., Vallinoto, M., Carneiro, M., Ferrand, N., Wilkins, M. R., Sequeira, F., Rollins, L. A., Holmes, E. C., Shine, R., & White, P. A. (2018). Draft genome assembly of the invasive cane toad, *Rhinella marina*. *Gigascience*, *7*(9), giy095. https://doi.org/10.1093/gigascience/giy095

Feder, J. L., Egan, S. P., & Nosil, P. (2012). The genomics of speciation-with-gene-flow. *Trends in Genetics*, *28*(7), 342–350. https://doi.org/10.1016/j.tig.2012.03.009

Firneno, T. J. Jr, O'Neill, J. R., Portik, D. M., Emery, A. H., Townsend, J. H., & Fujita, M. K. (2020). Finding complexity in complexes: Assessing the causes of mitonuclear discordance in a problematic species complex of Mesoamerican toads. *Molecular Ecology*, *29*(18), 3543–3559. https://doi.org/10.1111/mec.15496

Firneno, T. J. Jr, & Townsend, J. H. (2019). Evaluation of species boundaries in sympatric and parapatric populations of Mesoamerican toads. *Zoologica Scripta*, *48*(4), 454–465. https://doi.org/10.1111/zsc.12354

Fonseca, E. M., Gehara, M., Werneck, F. P., Lanna, F. M., Colli, G. R., Sites, J. W., Rodrigues, M. T., & Garda, A. A. (2018). Diversification with gene flow and niche divergence in a lizard species along the South American "diagonal of open formations". *Journal of Biogeography*, *45*(7), 1688–1700. https://doi.org/10.1111/jbi.13356

Fontenot, B. E., Makowsky, R., & Chippindale, P. T. (2011). Nuclear–mitochondrial discordance and gene flow in a recent radiation of toads. *Molecular Phylogenetics and Evolution*, *59*(1), 66–80. https://doi.org/10.1016/j.ympev.2010.12.018

Gamble, T., Simons, A. M., Colli, G. R., & Vitt, L. J. (2008). Tertiary climate change and the diversification of the Amazonian gecko genus *Gonatodes* (Sphaerodactylidae, Squamata). *Molecular Phylogenetics and Evolution*, *46*(1), 269–277. https://doi.org/10.1016/j.ympev.2007.08.013

Gehara, M., Crawford, A. J., Orrico, V. G. D., Rodríguez, A., Lötters, S., Fouquet, A., Barrientos, L. S., Brusquetti, F., De la Riva, I., Ernst, R., Urrutia, G. G., Glaw, F., Guayasamin, J. M., Hölting, M., Jansen, M., Kok, P. J. R., Kwet, A., Lingnau, R., Lyra, M., … Köhler, J. (2014). High levels of diversity uncovered in a widespread nominal taxon: continental phylogeography of the neotropical tree frog *Dendropsophus minutus*. *PLoS One*, *9*(9), e103958. https://doi.org/10.1371/journal.pone.0103958

Gehara, M., Garda, A. A., Werneck, F. P., Oliveira, E. F., da Fonseca, E. M., Camurugi, F., & Burbrink, F. T. (2017). Estimating synchronous demographic changes across populations using hABC and its application for a herpetological community from northeastern Brazil. *Molecular Ecology*, *26*(18), 4756–4771.

Graham, A. M., Lavretsky, P., Muñoz-Fuentes, V., Green, A. J., Wilson, R. E., & McCracken, K. G. (2018). Migration-selection balance drives genetic differentiation in genes associated with high-altitude function in the speckled teal (*Anas flavirostris*) in the Andes. *Genome Biology and Evolution*, *10*(1), 14–32. https://doi.org/10.1093/gbe/evx253

Green, R. E., Krause, J., Briggs, A. W., Maricic, T., Stenzel, U., Kircher, M., & Pääbo, S. (2010). A draft sequence of the Neandertal genome. *Science*, *328*(5979), 710–722. https://doi.org/10.1126/science.1188021

Guerra, C., Baldo, D., Rosset, S., Borteiro, C., & Kolenc, F. (2011). Advertisement and release calls in Neotropical toads of the *Rhinella granulosa* group and evidence of natural hybridization between *R. bergi* and *R. major* (Anura: Bufonidae). *Zootaxa*, *3092*, 26–42. https://doi.org/10.11646/zootaxa.3092.1.2

Guo, B., Lu, D., Liao, W. B., & Merilä, J. (2016). Genomewide scan for adaptive differentiation along altitudinal gradient in the Andrew's toad *Bufo andrewsi*. *Molecular Ecology*, *25*(16), 3884–3900. https://doi.org/10.1111/mec.13722

Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009). Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. *PLoS Genetics*, *5*(10), e1000695. https://doi.org/10.1371/journal.pgen.1000695

Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, *35*(2), 518–522. https://doi.org/10.1093/molbev/msx281

Jombart, T., & Ahmed, I. (2011). adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics*, *27*(21), 3070–3071.

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*, 94. https://doi.org/10.1186/1471-2156-11-94

Júnior, F. A. B., Caramaschi, U., & Haddad, C. F. B. (2004). Review of the *Bufo crucifer* species group, with descriptions of two new related species (Amphibia, Anura, Bufonidae). *Arquivos do Museu Nacional Museu Nacional*, *62*(3), 255–282. https://ia801001.us.archive.org/14/items/biostor-248672/biostor-248672.pdf

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., & Jermiin, L. S. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, *14*(6), 587–589. https://doi.org/10.1038/nmeth.4285

Lanfear, R., Calcott, B., Ho, S. Y. W., & Guindon, S. (2012). Partitionfinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, *29*(6), 1695–1701. https://doi.org/10.1093/molbev/mss020

Lanfear, R., Calcott, B., Kainer, D., Mayer, C., & Stamatakis, A. (2014). Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evolutionary Biology*, *14*, 82. https://doi.org/10.1186/1471-2148-14-82

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2017). PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, *34*(3), 772–773.

Lavretsky, P., Peters, J. L., Winker, K., Bahn, V., Kulikova, I., Zhuravlev, Y. N., Wilson, R. E., Barger, C., Gurney, K., & McCracken, K. G. (2016). Becoming pure: Identifying generational classes of admixed individuals within lesser and greater scaup populations. *Molecular Ecology*, *25*(3), 661–674. https://doi.org/10.1111/mec.13487

Leaché, A. D., Portik, D. M., Rivera, D., Rödel, M., Penner, J., Gvoždík, V., & Fujita, M. K. (2019). Exploring rain forest diversification using demographic model testing in the African foam-nest treefrog *Chiromantis rufescens*. *Journal of Biogeography*, *46*(12), 2706–2721. https://doi.org/10.1111/jbi.13716

Lisiecki, L. E., & Raymo, M. E. (2007). Plio-Pleistocene climate evolution: Trends and transitions in glacial cycle dynamics. *Quaternary Science Reviews*, *26*(1), 56–69. https://doi.org/10.1016/j.quascirev.2006.09.005

Liu, K., Wang, F., Chen, W., Tu, L., Min, M.-S., Bi, K., & Fu, J. (2010). Rampant historical mitochondrial genome introgression between two species of green pond frogs, *Pelophylax nigromaculatus* and *P. plancyi*. *BMC Evolutionary Biology*, *10*, 201. https://doi.org/10.1186/1471-2148-10-201

Lundberg, J. G., Marshall, L. G., Guerrero, J., Horton, B., Malabarba, M., & Wesselingh, F. (1998). The stage for Neotropical fish diversification: A history of tropical South American rivers. In L. R. Malabarba, R. E. Reis, R. P. Vari, Z. M. Lucena & C. A. S. Lucena (eds), *Phylogeny and Classification of Neotropical Fishes*, *27*, 13–48. Porto Alegre, Brazil: Museu de Ciências e Tecnologia, PUCRS.

Maciel, N. M., Collevatti, R. G., Colli, G. R., & Schwartz, E. F. (2010). Late Miocene diversification and phylogenetic relationships of the huge toads in the *Rhinella marina* (Linnaeus, 1758) species group (Anura: Bufonidae). *Molecular Phylogenetics and Evolution*, *57*(2), 787–797. https://doi.org/10.1016/j.ympev.2010.08.025

Malinsky, M., Matschiner, M., & Svardal, H. (2021). Dsuite - Fast D-statistics and related admixture evidence from VCF files. *Molecular Ecology Resources*, *21*, 584–595. https://doi.org/10.1111/1755-0998.13265

Malinsky, M., Svardal, H., Tyers, A. M., Miska, E. A., Genner, M. J., Turner, G. F., & Durbin, R. (2018). Whole-genome sequences of Malawi cichlids reveal multiple radiations interconnected by gene flow. *Nature Ecology & Evolution*, *2*(12), 1940–1955. https://doi.org/10.1038/s41559-018-0717-x

Mallet, J. (2005). Hybridization as an invasion of the genome. *Trends in Ecology & Evolution*, *20*(5), 229–237. https://doi.org/10.1016/j.tree.2005.02.010

Malone, J. H., & Fontenot, B. E. (2008). Patterns of reproductive isolation in toads. *PLoS One*, *3*(12), e3900. https://doi.org/10.1371/journal.pone.0003900

Marth, G. T., Czabarka, E., Murvai, J., & Sherry, S. T. (2004). The allele frequency spectrum in genome-wide human variation data reveals signals of differential demographic history in three large world populations. *Genetics*, *166*(1), 351–372. https://doi.org/10.1534/genetics.166.1.351

Martin, S. H., Dasmahapatra, K. K., Nadeau, N. J., Salazar, C., Walters, J. R., Simpson, F., & Jiggins, C. D. (2013). Genome-wide evidence for speciation with gene flow in *Heliconius* butterflies. *Genome Research*, *23*(11), 1817–1828.

Maya-Soriano, M. J., Holt, W. V., & Lloyd, R. E. (2012). Biobanked amphibian samples confirmed to species level using 16S rRNA DNA barcodes. *Biopreservation and Biobanking*, *10*(1), 22–28.

Mayr, E. (1963). *Animal species and evolution*. Harvard University Press. https://doi.org/10.4159/harvard.9780674865327

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). *Creating the CIPRES Science Gateway for inference of large phylogenetic trees. 2010 Gateway Computing Environments Workshop (GCE)*. https://doi.org/10.1109/gce.2010.5676129

Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, *32*(1), 268–274. https://doi.org/10.1093/molbev/msu300

Noonan, B. P., & Wray, K. P. (2006). Neotropical diversification: The effects of a complex history on diversity within the poison frog genus *Dendrobates*. *Journal of Biogeography*, *33*(6), 1007–1020. https://doi.org/10.1111/j.1365-2699.2006.01483.x

O'Connell, K. A., Prates, I., Scheinberg, L. A., Mulder, K. P., & Bell, R. C. (2021). Speciation and secondary contact in a fossorial island endemic, the São Tomé caecilian. *Molecular Ecology*, *30*(12), 2859–2871. https://doi.org/10.1111/mec.15928

Oliveira, E. F., Gehara, M., São-Pedro, V. A., Costa, G. C., Burbrink, F. T., Colli, G. R., Rodrigues, M. T., & Garda, A. A. (2018). Phylogeography of Muller's termite frog suggests the vicariant role of the Central Brazilian Plateau. *Journal of Biogeography*, *45*(11), 2508–2519. https://doi.org/10.1111/jbi.13427

Patterson, N., Moorjani, P., Luo, Y., Mallick, S., Rohland, N., Zhan, Y., Genschoreck, T., Webster, T., & Reich, D. (2012). Ancient admixture in human history. *Genetics*, *192*(3), 1065–1093. https://doi.org/10.1534/genetics.112.145037

Pereyra, M. O., Baldo, D., Blotto, B. L., Iglesias, P. P., Thome, M. T. C., Haddad, C. F. B., & Faivovich, J. (2016). Phylogenetic relationships of toads of the *Rhinella granulosa* group (Anura: Bufonidae): A molecular perspective with comments on hybridization and introgression. *Cladistics*, *32*, 36–53.

Pereyra, M. O., Blotto, B. L., Baldo, D., Chaparro, J. C., Ron, S. R., Elias-Costa, A. J., Iglesias, P. P., Venegas, P. J., C. Thomé, M. T., Ospina-Sarria, J. J., Maciel, N. M., Rada, M., Kolenc, F., Borteiro, C., Rivera-Correa, M., Rojas-Runjaic, F. J. M., Moravec, J., De La Riva, I., Wheeler, W. C., … Faivovich, J. (2021). Evolution in the Genus *Rhinella*: A total evidence phylogenetic analysis of Neotropical true toads (Anura: Bufonidae). *Bulletin of the American Museum of Natural History*, *447*(1), 1–156. https://doi.org/10.1206/0003-0090.447.1.1

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One*, *7*(5), e37135. https://doi.org/10.1371/journal.pone.0037135

Phuong, M. A., Bi, K., & Moritz, C. (2017). Range instability leads to cytonuclear discordance in a morphologically cryptic ground squirrel

species complex. *Molecular Ecology*, *26*(18), 4743–4755. https://doi.org/10.1111/mec.14238

Pirani, R. M., Peloso, P. L. V., Prado, J. R., Polo, É. M., Knowles, L. L., Ron, S. R., Rodrigues, M. T., Sturaro, M. J., & Werneck, F. P. (2020). Diversification history of clown tree frogs in Neotropical rainforests (Anura, Hylidae, *Dendropsophus leucophyllatus* group). *Molecular Phylogenetics and Evolution*, *150*, 106877. https://doi.org/10.1016/j.ympev.2020.106877

Portik, D. (2016). *Phylo_Wrapper_Scripts*. Retrieved from https://github.com/dportik/Phylo_Wrapper_Scripts

Portik, D. M., Leaché, A. D., Rivera, D., Barej, M. F., Burger, M., Hirschfeld, M., Rödel, M.-O., Blackburn, D. C., & Fujita, M. K. (2017). Evaluating mechanisms of diversification in a Guineo-Congolian tropical forest frog using demographic model selection. *Molecular Ecology*, *26*(19), 5245–5263. https://doi.org/10.1111/mec.14266

Potter, S., Bragg, J. G., Peter, B. M., Bi, K., & Moritz, C. (2016). Phylogenomics at the tips: Inferring lineages and their demographic history in a tropical lizard, Carlia Amax. *Molecular Ecology*, *25*(6), 1367–1380.

Prado, C. P. A., Haddad, C. F. B., & Zamudio, K. R. (2012). Cryptic lineages and Pleistocene population expansion in a Brazilian Cerrado frog. *Molecular Ecology*, *21*, 921–941. https://doi.org/10.1111/j.1365-294X.2011.05409.x

Pramuk J. B., Robertson T., Sites J. W., Noonan B. P. (2008). Around the world in 10 million years: biogeography of the nearly cosmopolitan true toads (Anura: Bufonidae). *Global Ecology and Biogeography*, *17*(1), 72–83. http://dx.doi.org/10.1111/j.1466-8238.2007.00348.x

Prates, I., Rivera, D., Rodrigues, M. T., & Carnaval, A. C. (2016). A mid-Pleistocene rainforest corridor enabled synchronous invasions of the Atlantic Forest by Amazonian anole lizards. *Molecular Ecology*, *25*(20), 5174–5186.

Prates, I., Xue, A. T., Brown, J. L., Alvarado-Serrano, D. F., Rodrigues, M. T., Hickerson, M. J., & Carnaval, A. C. (2016). Inferring responses to climate dynamics from historical demography in neotropical forest lizards. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(29), 7978–7985. https://doi.org/10.1073/pnas.1601063113

Rabosky, D. L. (2016). Reproductive isolation and the causes of speciation rate variation in nature. *Biological Journal of the Linnean Society*, *118*(1), 13–25. https://doi.org/10.1111/bij.12703

Rambaut A., Drummond A. J, Xie D., Baele G., Suchard M. A. (2018). Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Systematic Biology*, *67*(5), 901–904. http://dx.doi.org/10.1093/sysbio/syy032

Ribeiro-Júnior, M. A. (2015). Catalogue of distribution of lizards (Reptilia: Squamata) from the Brazilian Amazonia. I. Dactyloidae, Hoplocercidae, Iguanidae, Leiosauridae, Polychrotidae, Tropiduridae. *Zootaxa*, *3983*(1), 1–110.

Ribeiro-Júnior, M. A., & Amaral, S. (2017). Catalogue of distribution of lizards (Reptilia: Squamata) from the Brazilian Amazonia. IV. Alopoglossidae, Gymnophthalmidae. *Zootaxa*, *4269*(2), 151–196.

Rockney, H. J., Ofori-Boateng, C., Porcino, N., & Leaché, A. D. (2015). A comparison of DNA barcoding markers in West African frogs. *African Journal of Herpetology*, *64*(2), 135–147. https://doi.org/10.1080/21564574.2015.1114530

Ronquist F., Teslenko M., van der Mark P., Ayres D. L., Darling A., Höhna S., Larget B., Liu L., Suchard M. A., Huelsenbeck J. P. (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology*, *61*(3), 539–542. http://dx.doi.org/10.1093/sysbio/sys029

Sanchíz, B. (1998). Part 4. Salientia. In *Handbuch der Paläoherpetologie/Encyclopedia of Paleoherpetology*. München: Dr. Friedrich Pfeil.

Sambrook, J., & Russell, D. W. (2006). Purification of nucleic acids by extraction with phenol:chloroform. *Cold Spring Harbor Protocols*, *2006*(1), pdb.prot4455. https://doi.org/10.1101/pdb.prot4455

Sequeira, F., Sodré, D., Ferrand, N., Bernardi, J. A. R., Sampaio, I., Schneider, H., & Vallinoto, M. (2011). Hybridization and massive mtDNA unidirectional introgression between the closely related Neotropical toads *Rhinella marina* and *R. schneideri* inferred from mtDNA and nuclear markers. *BMC Evolutionary Biology*, *11*(1), 264. https://doi.org/10.1186/1471-2148-11-264

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Streicher, J. W., Devitt, T. J., Goldberg, C. S., Malone, J. H., Blackmon, H., & Fujita, M. K. (2014). Diversification and asymmetrical gene flow across time and space: Lineage sorting and hybridization in polytypic barking frogs. *Molecular Ecology*, *23*(13), 3273–3291. https://doi.org/10.1111/mec.12814

Thomé, M. T. C., Zamudio, K. R., Giovanelli, J. G. R., Haddad, C. F. B., Baldissera, F. A. Jr, & Alexandrino, J. (2010). Phylogeography of endemic toads and post-Pliocene persistence of the Brazilian Atlantic Forest. *Molecular Phylogenetics and Evolution*, *55*(3), 1018–1031. https://doi.org/10.1016/j.ympev.2010.02.003

Thomé, M. T. C., Zamudio, K. R., Haddad, C. F. B., & Alexandrino, J. (2012). Delimiting genetic units in Neotropical toads under incomplete lineage sorting and hybridization. *BMC Evolutionary Biology*, *12*, 242. https://doi.org/10.1186/1471-2148-12-242

Vallinoto, M., Sequeira, F., Sodre, D., Bernardi, J. A. R., Sampaio, I., & Schneider, H. (2010). Phylogeny and biogeography of the *Rhinella marina* species complex (Amphibia, Bufonidae) revisited: Implications for Neotropical diversification hypotheses. *Zoologica Scripta*, *39*(2), 128–140. https://doi.org/10.1111/j.1463-6409.2009.00415.x

Vasconcellos, M. M., Colli, G. R., Weber, J. N., Ortiz, E. M., Rodrigues, M. T., & Cannatella, D. C. (2019). Isolation by instability: Historical climate change shapes population structure and genomic divergence of treefrogs in the Neotropical Cerrado savanna. *Molecular Ecology*, *28*(7), 1748–1764. https://doi.org/10.1111/mec.15045

Venâncio N. M., Antonio de Freitas M., Abegg A. D., de Kokubum M. N. C. (2017). First record of Rhinella poeppigii (Tschudi, 1845) in Brazil (Anura, Bufonidae). *Check List*, *13*(6), 747–750. http://dx.doi.org/10.15560/13.6.747

Vences, M., Thomas, M., van der Meijden, A., Chiari, Y., & Vieites, D. R. (2005). Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in Zoology*, *2*(1), 5.

Venegas, P. J., & Ron, S. R. (2014). First records of *Rhinella poeppigii* (Tschudi 1845) from Ecuador, with a distribution map (Anura: Bufonidae). *Herpetology Notes*, *7*, 713–716.

Wang, X., Edwards, R. L., Auler, A. S., Cheng, H., Kong, X., Wang, Y., Cruz, F. W., Dorale, J. A., & Chiang, H.-W. (2017). Hydroclimate changes across the Amazon lowlands over the past 45,000 years. *Nature*, *541*(7636), 204–207. https://doi.org/10.1038/nature20787

Werneck, F. P. (2011). The diversification of eastern South American open vegetation biomes: Historical biogeography and perspectives. *Quaternary Science Reviews*, *30*(13), 1630–1648. https://doi.org/10.1016/j.quascirev.2011.03.009

Werneck, F. P., Leite, R. N., Geurgas, S. R., & Rodrigues, M. T. (2015). Biogeographic history and cryptic diversity of saxicolous Tropiduridae lizards endemic to the semiarid Caatinga. *BMC Evolutionary Biology*, *15*, 94. https://doi.org/10.1186/s12862-015-0368-3

Wesselingh, F. P., & Salo, J. A. (2006). A Miocene perspective on the evolution of the Amazonian biota. *Scripta Geologica*, *133*, 439–458.

Winter, D. J. (2012). MMOD: An R library for the calculation of population differentiation statistics. *Molecular Ecology Resources*, *12*(6), 1158–1160.

Yeaman, S., & Whitlock, M. C. (2011). The genetic architecture of adaptation under migration-selection balance: The genetic architecture of local adaptation. *Evolution*, *65*(7), 1897–1911. https://doi.org/10.1111/j.1558-5646.2011.01269.x

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